

Amendments to the Claims

1. (original) A catalytic domain of BACE or a form of BACE that is suitable for crystallization with the correct disulphide bonding that eliminates the need for refolding and/or an apo-BACE crystal or an apo-BACE crystal that can be soaked to give complexes and/or a crystalline form of BACE having crystals that are grown at or near the physiological pH of the enzyme or between about pH 5.6 and about pH 5.8 and/or a BACE crystal having a space group of C2 and/or a BACE crystal having cell dimensions of $a = 236.63 \text{ \AA}$ or $236.63 \text{ \AA} \pm \text{standard deviation}$ (0.2 \AA) or $236.63 \text{ \AA} \pm 3.0 \text{ \AA}$, $b = 105.02 \text{ \AA}$ or $105.02 \text{ \AA} \pm \text{standard deviation}$ (0.2 \AA) or $105.02 \text{ \AA} \pm 3.0 \text{ \AA}$, and $c = 62.59 \text{ \AA}$ or $62.59 \text{ \AA} \pm \text{standard deviation}$ (0.2 \AA) or $62.59 \text{ \AA} \pm 3.0 \text{ \AA}$ and $\beta = 101.32^\circ$ or $101.32^\circ \pm \text{standard deviation}$ (0.2°) or between 101° and 108° with the asymmetric unit of the crystal containing three copies of BACE or cell dimensions $a = 238.3 \text{ \AA}$ or $238.3 \text{ \AA} \pm \text{standard deviation}$ (0.2 \AA) or $238.3 \text{ \AA} \pm 3.0 \text{ \AA}$, $b = 107.4 \text{ \AA} \pm \text{standard deviation}$ (0.2 \AA) or $107.4 \text{ \AA} \pm 3.0 \text{ \AA}$, and $c = 60.4 \text{ \AA}$ or $60.4 \text{ \AA} \pm \text{standard deviation}$ (0.2 \AA) or $60.4 \text{ \AA} \pm 3.0 \text{ \AA}$ and $\beta = 101.89^\circ$ or $101.89^\circ \pm \text{standard deviation}$ (0.2°) or between 101° and 108° and/or having an X-ray diffraction pattern corresponding to or resulting from any or all of the foregoing and/or having an X-ray diffraction pattern corresponding to or resulting from any or all of the foregoing; and/or having a space group transition from C2 to $P2_1$ together with an increase in the number of copies of the molecule in the asymmetric unit, while the cell dimensions and the packing of the $P2_1$ form are closely related to those of the C2 crystal form, on soaking the apo-BACE crystal with a ligand; and/or a BACE crystal having a resolution better than 3 \AA ; and/or a BACE crystal having the structure defined by the co-ordinates of Table 5.
2. (original) A BACE crystal having the structure defined by the co-ordinates of Table 5.
3. (original) An apo-BACE crystal grown at or near the physiological pH of the enzyme.
4. (original) An apo-BACE crystal or an apo-BACE crystal that can be soaked to give complexes.

5. (original) A crystalline form of BACE or a functional portion thereof having crystals that are grown at or near the physiological pH of the enzyme.
6. (original) The crystalline form of BACE or functional portion thereof of claim 6 wherein the crystals are grown at a pH between about pH 5.6 and about pH 5.8.
7. (original) A crystalline form of BACE or a functional portion thereof having a space group of C2 and cell dimensions of $a = 236.63 \text{ \AA}$ or $236.63 \text{ \AA} \pm \text{standard deviation } (0.2 \text{ \AA})$ $236.63 \text{ \AA} \pm 3.0 \text{ \AA}$, $b = 105.02 \text{ \AA}$ or $105.02 \text{ \AA} \pm \text{standard deviation } (0.2 \text{ \AA})$ or $105.02 \text{ \AA} \pm 3.0 \text{ \AA}$, and $c = 62.59 \text{ \AA}$ or $62.59 \text{ \AA} \pm \text{standard deviation } (0.2 \text{ \AA})$ or $62.59 \text{ \AA} \pm 3.0 \text{ \AA}$ and $\beta = 101.32^\circ$ or $101.32^\circ \pm \text{standard deviation } (0.2^\circ)$ or between 101° and 108° with the asymmetric unit of the crystal containing three copies of BACE or cell dimensions $a = 238.3 \text{ \AA}$ or $238.3 \text{ \AA} \pm \text{standard deviation } (0.2 \text{ \AA})$ or $238.3 \text{ \AA} \pm 3.0 \text{ \AA}$, $b = 107.4 \text{ \AA}$ or $107.4 \text{ \AA} \pm \text{standard deviation } (0.2 \text{ \AA})$ or $107.4 \text{ \AA} \pm 3.0 \text{ \AA}$, and $c = 60.4 \text{ \AA}$ or $60.4 \text{ \AA} \pm \text{standard deviation } (0.2 \text{ \AA})$ or $60.4 \text{ \AA} \pm 3.0 \text{ \AA}$ and $\beta = 101.89^\circ$ or $101.89^\circ \pm \text{standard deviation } (0.2^\circ)$ or between 101° and 108° and/or having an X-ray diffraction pattern corresponding to or resulting from any or all of the foregoing and/or having an X-ray diffraction pattern corresponding to or resulting from any or all of the foregoing and/or having a space group transition from C2 to P2₁ together with an increase in the number of copies of the molecule in the asymmetric unit, while the cell dimensions and the packing of the P2₁ form are closely related to those of the C2 crystal form, on soaking the apo-BACE crystal with a ligand.
8. (original) A crystalline form of BACE or a functional portion thereof that has an active site containing one or more ligands other than the natural substrate or the substrate that occurs naturally or physiologically within the active site.
9. (original) A method for ligand screening or identification comprising exposing the BACE crystals of any one of claims 2-8 to one or more test samples, and determining whether a ligand-BACE complex is formed.

10. (original) The method of claim 9 wherein the BACE protein or functional portion thereof is exposed to the test samples by co-crystallizing the BACE protein or functional portion thereof in the presence of the one or more test samples.

11. (original) The method of claim 9 wherein the BACE of claims 2-8 is soaked in a solution of one or more test samples.

12. (original) A computer-assisted method for identifying or designing potential ligands to fit within the catalytic domain of BACE or a functional portion thereof:

comprising using a programmed computer comprising a processor, a data storage system, an input device, and an output device, the steps of: (a) inputting into the programmed computer through said input device data comprising the three-dimensional co-ordinates of a subset of the atoms in the BACE catalytic domain, optionally with structural information from ligand-BACE complexes, thereby generating a data set; (b) comparing, using said processor, said data set to a computer database of chemical structures stored in said computer data storage system; (c) selecting from said database, using computer methods, chemical structures having a portion that is structurally similar to said data set; (d) constructing, using computer methods, a model of a chemical structure having a portion that is structurally similar to said data set and (e) outputting to said output device the selected chemical structures having a portion similar to said data set; and optionally synthesizing one or more of the selected chemical structures; and further optionally contacting said synthesized selected chemical structure with BACE to ascertain whether said synthesized chemical structure is a ligand that fits within the catalytic domain of BACE and/or inhibits BACE; or,

comprising: providing the structure of BACE as defined by the co-ordinates of Table 5, providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the structure of the BACE of Table 5; or,

comprising: providing the co-ordinates of at least two atoms of Table 5 of BACE ("selected co-ordinates"), providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the selected co-ordinates of BACE; or,

comprising: providing the co-ordinates of at least a sub-domain of BACE, providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the sub-domain of BACE;

said method optionally further comprising: obtaining or synthesizing the chemical structure or candidate modulator and contacting the chemical structure or candidate modulator with BACE to determine the ability of the chemical structure or candidate to interact with BACE; or obtaining or synthesizing the chemical structure or candidate modulator and forming a complex of BACE and said chemical structure or candidate modulator, and analyzing the complex to determine the ability of said chemical structure or candidate modulator to interact with BACE.

13. (original) A compound having a chemical structure selected using the methods of claims 9-12, said compound being a modulator of BACE.

14. (original) A BACE protein or functional portion thereof comprising amino acid sequences of the catalytic domain that crystallize to the crystalline structure of claim 7, or to a structure that mimics that crystalline structure.

15. (original) A BACE protein or functional portion thereof which, when compared to wild-type BACE or BACE of Genbank accession P56817 has one or more mutations or truncations to prevent glycosylation or facilitate crystallization and/or the growth of ordered, well-diffracting crystals.

16. (original) The BACE protein or functional portion thereof of claim 15, which when compared with Genbank accession P56817 has one or more of : a mutation at amino acid ("aa") 153, a mutation at aa 172, a mutation at aa 223, a mutation at aa 354, and one or more truncations.

17. (original) The BACE protein or functional portion thereof of claim 16 wherein each of the mutations is asparagine to glutamine.

18. (original) The BACE protein or functional portion thereof of claim 16 wherein the truncation results in a BACE extending from Thr 22 to Ser 453, with reference to Genbank Accession P56817.
19. (original) The BACE protein or functional portion thereof of claim 16 wherein all of the mutations are present and each is asparagine to glutamine and there is a truncation resulting in a BACE extending from Thr 22 to Ser 453, with reference to Genbank Accession P56817.
20. (original) The BACE protein or functional portion thereof of any one of claims 14-19 further including any one or more of: a tag to facilitate purification; a non-BACE signal sequence to facilitate or increase secretion of the protein into cell culture medium; and a tag to allow differentiation of species arising from incomplete pro-peptide cleavage.
21. (original) The BACE protein or functional portion thereof of claim 20 wherein the tag to facilitate purification is a HIS tag, the non-BACE signal sequence is a baculovirus signal sequence, and the tag to allow differentiation of species is a FLAG tag.
22. (original) The BACE protein or functional portion thereof of claim 21 wherein all of the tag to facilitate purification, the non-BACE signal sequence and the tag to allow differentiation are present.
23. (original) A BACE protein or functional portion thereof containing any one or more of: a tag to facilitate purification; a non-BACE signal sequence to facilitate or increase secretion of the protein into cell culture medium; and a tag to allow differentiation of species arising from incomplete pro-peptide cleavage.
24. (original) The BACE protein or functional portion thereof of claim 23 wherein the tag to facilitate purification is a HIS tag, the non-BACE signal sequence is a baculovirus signal sequence, and the tag to allow differentiation of species is a FLAG tag.

25. (original) The BACE protein or functional portion thereof of claim 24 wherein all of the tag to facilitate purification, the non-BACE signal sequence and the tag to allow differentiation are present.
26. (original) An isolated nucleic acid molecule encoding a BACE protein or functional portion thereof of any of claims 14-25 or a functional portion thereof.
27. (original) The isolated nucleic acid molecule of claim 26 that has a reduced GC content via silent mutations from nucleotide sequences derived from wild-type BACE that would also encode the BACE protein.
28. (original) A vector or cell comprising or expressing the nucleic acid molecule of claim 26.
29. (original) A vector or cell comprising or expressing the nucleic acid molecule of claim 27.
30. (original) The vector or cell of claim 28 which is a viral vector or a bacterial vector or a mammalian cell or a DNA plasmid.
31. (original) The vector or cell of claim 29 which is a viral vector or a bacterial vector or a mammalian cell or a DNA plasmid.
32. (original) The vector or cell of claims 30 or 31 which is a baculovirus vector or an insect cell.
33. (original) The vector or cell of claim 26 further including a nucleic acid molecule encoding an enhancer that enhances in the particular vector or cell system the total amount of BACE produced and/or increases the fraction of processed protein.

34. (original) The vector or cell of claim 27 further including a nucleic acid molecule encoding an enhancer that enhances in the particular vector or cell system the total amount of BACE produced and/or increases the fraction of processed protein.
35. (original) The vector or cell of claims 33 or 34 wherein the enhancer is a prohormone convertase.
36. (original) The vector or cell of claim 35 wherein the prohormone convertase is furin.
37. (original) A vector or cell comprising a nucleic acid molecule encoding a BACE protein or functional portion thereof and a nucleic acid molecule encoding an enhancer that enhances in the particular vector or cell system the total amount of BACE produced and/or increases the fraction of processed protein.
38. (original) The vector or cell of claim 37 wherein the enhancer is a prohormone convertase.
39. (original) A kit for producing the vector or cell of claim 37 containing separately packaged nucleic acid molecules comprising (i) a BACE-protein encoding nucleic acid molecule and (ii) a nucleic acid molecule encoding the enhancer.
40. (original) A method for obtaining a BACE protein comprising expressing a nucleic acid molecule according to any of claims 26 or 27 or the nucleic acid molecule of the vector or cell of any of claims 28 to 34 or 37.
41. (original) A method for obtaining a BACE protein comprising expressing the nucleic acid molecule of the vector or cell of claim comprising expressing in a vector or cell the nucleic acid molecules of the kit of claim 39.
42. (original) A method for crystallizing a BACE protein or functional portion thereof comprising dissolving a BACE protein according to any one of claims 14-25 in a suitable solvent and crystallizing the same either in the presence or absence of an inhibitor; wherein said method

optionally further includes producing the BACE recombinantly or by expression thereof by a vector, recovering the BACE so produced, and growing crystals from the recovered BACE.

43. (original) The method of claim 42 wherein the inhibitor is OM99-2.

44. (original) A method for determining the crystal structure of a BACE protein or functional portion thereof comprising obtaining crystals of a BACE protein according to any one of claims 14-25 and obtaining an x-ray diffraction pattern thereof.

45. (original) A method for ligand screening and design or identification comprising exposing the BACE crystals of a BACE protein or functional portion thereof to one or more test samples, and determining whether a ligand-BACE complex is formed; wherein the BACE or functional portion thereof has an unoccupied active site and is as claimed in any one of claims 5-8.

46. (original) The method of claim 45 wherein the BACE is exposed to the test samples by either co-crystallizing the BACE or functional portion thereof in the presence of the one or more test samples or soaking the BACE or a functional portion thereof in a solution of one or more test samples.

47. (original) A computer-assisted method for identifying or designing potential ligands to fit within the catalytic domain of BACE or a functional portion thereof:

comprising using a programmed computer comprising a processor, a data storage system, an input device, and an output device, the steps of: (a) inputting into the programmed computer through said input device data comprising the three-dimensional co-ordinates of a subset of the atoms in the BACE catalytic domain or functional portion thereof of any one of claims 5-8, optionally with structural information from ligand-BACE complexes, thereby generating a data set; (b) comparing, using said processor, said data set to a computer database of chemical structures stored in said computer data storage system; (c) selecting from said database, using computer methods, chemical structures having a portion that is structurally similar to said data set; (d) constructing, using computer methods, a model of a chemical structure having a portion that is structurally similar to said data set and (e) outputting to said output device the selected chemical

structures having a portion similar to said data set; and optionally synthesizing one or more of the selected chemical structures; and further optionally contacting said synthesized selected chemical structure with BACE to ascertain whether said synthesized chemical structure is a ligand that fits within the catalytic domain of BACE and/or inhibits BACE; or,

comprising: providing the structure of BACE as defined by the co-ordinates of Table 5, providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the structure of the BACE of Table 5; or,

comprising: providing the co-ordinates of at least two atoms of Table 5 of BACE ("selected co-ordinates"), providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the selected co-ordinates of BACE; or,

comprising: providing the co-ordinates of at least a sub-domain of BACE, providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the sub-domain of BACE;

said method optionally further comprising: obtaining or synthesizing the chemical structure or candidate modulator and contacting the chemical structure or candidate modulator with BACE to determine the ability of the chemical structure or candidate to interact with BACE; or obtaining or synthesizing the chemical structure or candidate modulator and forming a complex of BACE and said chemical structure or candidate modulator, and analyzing the complex to determine the ability of said chemical structure or candidate modulator to interact with BACE.

48. (original) A ligand identified in any of the methods of claims 45-47.

49. (original) An assay comprising a BACE protein or functional portion thereof of any one of claims 14-25, and means to determine whether a compound is a modulator of BACE.

50. (original) An antibody elicited by a BACE protein or functional portion thereof of any one of claims 14-25.

51. (original) An inhibitor of a BACE protein or functional portion thereof of any one of claims 14-25.

52. (original) A composition comprising the inhibitor of claim 51.
53. (original) A composition comprising the ligand of claim 48.
54. (original) A composition comprising the ligand of claim 13.
55. (original) A composition comprising a product from the assay of claim 49.
56. (original) A method for inhibiting BACE or the production of A β or fragments thereof or treating AD in an individual in need thereof comprising administering an inhibitor of a BACE protein or functional portion thereof as claimed in claim 51.
57. (original) A method for inhibiting BACE or the production of A β or fragments thereof or treating AD in an individual in need thereof comprising administering a ligand of claim 13.
58. (original) A method for inhibiting BACE or the production of A β or fragments thereof or treating AD in an individual in need thereof comprising administering a ligand of claim 48.
59. (currently amended) A BACE which comprises an amino acid sequence of SEQ ID NO: ~~5~~ 31 or an amino acid sequence having greater than 98.8% identity with SEQ ID NO: ~~5~~ 31.
60. (currently amended) The BACE of claim 59 having the amino acid sequence of SEQ ID NO: ~~5~~ 31.
61. (original) A nucleic acid molecule encoding the BACE of claim 59 or 60.
62. (currently amended) An isolated nucleic acid molecule comprising a sequence of SEQ ID NO: ~~4~~ 32 or [[10]] encoding a polypeptide sequence of SEQ ID 72 or a sequence having greater than 95.6% identity with SEQ ID NO: ~~4~~ 32 or [[10]] the nucleic acid encoding a polypeptide sequence of SEQ ID 72.

63. (currently amended) The isolated nucleic acid molecule of claim 62 having the sequence of SEQ ID NO:4 32.

64. (currently amended) The isolated nucleic acid molecule of claim 63 ~~having the sequence~~ encoding a polypeptide sequence of SEQ ID NO:10 72.

65. (original) A vector or cell comprising the isolated nucleic acid molecule of any one of claims 62-64.

66. (original) The vector or cell of claim 65 which is a baculovirus vector or an insect cell.

67. (original) An inhibitor of the BACE of any one of claims 59 or 60.

68. (original) An antibody elicited by the BACE of any one of claims 59 or 60.

69. (original) A method for ligand screening and design or identification comprising exposing the BACE crystals of a BACE protein or functional portion thereof to one or more test samples, and determining whether a ligand-BACE complex is formed; wherein the BACE or functional portion thereof has an unoccupied active site and is as claimed in any one of claims 59 or 60.

70. (original) The method of claim 69 wherein the BACE is exposed to the test samples by either co-crystallizing the BACE or functional portion thereof in the presence of the one or more test samples or soaking the BACE or a functional portion thereof in a solution of one or more test samples.

71. (original) A computer-assisted method for identifying or designing potential ligands to fit within the catalytic domain of BACE or a functional portion thereof:

comprising using a programmed computer comprising a processor, a data storage system, an input device, and an output device, the steps of: (a) inputting into the programmed computer through said input device data comprising the three-dimensional co-ordinates of a subset of the atoms in the BACE catalytic domain or functional portion thereof of any one of claims 59 or 60,

optionally with structural information from ligand-BACE complexes, thereby generating a data set; (b) comparing, using said processor, said data set to a computer database of chemical structures stored in said computer data storage system; (c) selecting from said database, using computer methods, chemical structures having a portion that is structurally similar to said data set; (d) constructing, using computer methods, a model of a chemical structure having a portion that is structurally similar to said data set and (e) outputting to said output device the selected chemical structures having a portion similar to said data set; and optionally synthesizing one or more of the selected chemical structures; and further optionally contacting said synthesized selected chemical structure with BACE to ascertain whether said synthesized chemical structure is a ligand that fits within the catalytic domain of BACE and/or inhibits BACE; or,

comprising: providing the structure of BACE as defined by the co-ordinates of Table 5, providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the structure of the BACE of Table 5; or,

comprising: providing the co-ordinates of at least two atoms of Table 5 of BACE ("selected co-ordinates"), providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the selected co-ordinates of BACE; or,

comprising: providing the co-ordinates of at least a sub-domain of BACE, providing the structure of a candidate modulator molecule, and fitting the structure of the candidate to the sub-domain of BACE;

said method optionally further comprising: obtaining or synthesizing the chemical structure or candidate modulator and contacting the chemical structure or candidate modulator with BACE to determine the ability of the chemical structure or candidate to interact with BACE; or obtaining or synthesizing the chemical structure or candidate modulator and forming a complex of BACE and said chemical structure or candidate modulator, and analyzing the complex to determine the ability of said chemical structure or candidate modulator to interact with BACE.

72. (original) A ligand identified in any of the methods of claims 68-71.

73. (original) An assay comprising a BACE protein or functional portion thereof of any one of claims 58 or 59, and means to determine whether a compound is a modulator of BACE.

74. (original) A composition comprising the inhibitor of claim 67.
75. (original) A composition comprising the ligand of claim 72.
76. (original) A composition comprising a product from the assay of claim 73.
77. (original) A method for inhibiting BACE or the production of A β or fragments thereof or treating AD in an individual in need thereof comprising administering an inhibitor of a BACE protein or functional portion thereof as claimed in claim 67.
78. (original) A method for inhibiting BACE or the production of A β or fragments thereof or treating AD in an individual in need thereof comprising administering a ligand of claim 72.
79. (original) Use of an inhibitor of a BACE protein or functional portion thereof as claimed in claim 51 for preparing a composition or medicament for inhibiting BACE or the production of A β or fragments thereof or treating AD in an individual in need thereof.
80. (original) Use of an inhibitor of a BACE protein or functional portion thereof as claimed in claim 13 for preparing a composition or medicament for inhibiting BACE or the production of A β or fragments thereof or treating AD in an individual in need thereof.
81. (original) Use of an inhibitor of a BACE protein or functional portion thereof as claimed in claim 48 for preparing a composition or medicament for inhibiting BACE or the production of A β or fragments thereof or treating AD in an individual in need thereof.
82. (original) Use of an inhibitor of a BACE protein or functional portion thereof as claimed in claim 51 for use in therapy.
83. (original) Use of an inhibitor of a BACE protein or functional portion thereof as claimed in claim 67 for preparing a composition or medicament for inhibiting BACE or the production of A β or fragments thereof or treating AD in an individual in need thereof.

84. (original) Use of an inhibitor of a BACE protein or functional portion thereof as claimed in claim 72 for preparing a composition or medicament for inhibiting BACE or the production of A β or fragments thereof or treating AD in an individual in need thereof.

85. (original) A computer system for generating structures or performing rational compound or drug design for BACE or complexes of BACE with a potential modulator, the system containing either: atomic co-ordinate data according to Table 5, said data defining the three-dimensional structure of BACE or at least one sub-domain thereof, or structure factor data for BACE, said structure factor data being derivable from the atomic co-ordinate data of Table 5.

86. (original) A computer readable media with either: atomic co-ordinate data according to Table 5, said data defining the three-dimensional structure of BACE or at least one sub-domain thereof, or structure factor data for BACE, said structure factor data being derivable from the atomic co-ordinate data of Table 5.

87. (original) A method of doing business comprising providing to a user the computer system of claim 85 or the computer readable media of claim 83 or the three-dimensional structure of BACE or at least one sub-domain thereof, or structure factor data for BACE, said structure factor data being derivable from the atomic co-ordinate data of Table 5.